

ADAMTS POLYPEPTIDES, NUCLEIC ACIDS ENCODING THEM, AND USES THEREOFField of Invention

The present invention relates to a member of the family of proteins known as ADAMTS.

5

Background Of The Invention

ADAMTS proteins exhibit characteristics of the ADAM (A Disintegrin And Metalloprotease) family of metalloproteases, and in addition contain a thrombospondin domain (TS). The prototypic ADAMTS was identified in mouse, found to be expressed in heart and kidney and upregulated by proinflammatory stimuli (K. Kuno et al., *Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as a inflammation associated gene*, 272 *Journal of Biological Chemistry* 556 (January 1997)). To date eight members are recognized by the HUGO database (<http://www.gene.ucl.ac.uk/users/hester/adamts>.) with sequence information available for all but two of these. Members of this family have the ability to degrade aggrecan, a high molecular weight proteoglycan which provides cartilage with important mechanical properties and which is lost during the development of arthritis.

Aggrecanase activity has been demonstrated for several ADAMTS proteins (See, e.g., M.D. Tortorella, *Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins*, 284 *Science* 1664 (June 1999); I. Abbaszade, *Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family*, 274 *Journal of Biological Chemistry* 23443 (August 1999)). In addition to aggrecanase activity, ADAMTS-4 was shown to cleave another proteoglycan, brevican, found expressed predominately in the central nervous system (Matthews et al., *Brain-enriched hyaluronan binding (BEHAB)/Brevican cleavage in a glioma cell line is mediated by a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family member*, 275 *Journal of Biological Chemistry* 22695 (July 2000)). This activity was speculated to play a role in the invasiveness of glioma. Additional activities of ADAMTS-4 are proposed as its expression was induced in rat astrocytes treated with beta-amyloid, suggesting a role in Alzheimer's disease (Sato et al., *ADAMTS-4 is transcriptionally induced in beta-amyloid treated rat astrocytes*, 289 *Neuroscience Letters* 177 (2000)). Other ADAMTS proteins are reported to exhibit antiangiogenic (See, e.g. F. Vazquez et al., *METH-1, A human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with Angio-inhibitory activity*, 274 *Journal of Biological Chemistry* 23349 (August 1999)) and/or procollagen processing activities (A. Colige et al., *cDNA cloning and expression of bovine procollagen I N-proteinase: a new member of the superfamily of zinc-metalloproteinase with binding sites for cells and other matrix components*, 94 *Proceedings of the National Academy of Sciences of the United States of America* 2374 (March 1997)). Additional roles for ADAMTS-1 in fertility and organ

EXPRESS MAIL NO. EL446402267US

development, particularly with respect to the urogenital system, were implicated by gene knockout experiments in mice (Shindo et al., *ADAMTS-1: a metalloprotease-disintegrin essential for normal growth, fertility, and organ morphology and function*, 105 Journal of Clinical Investigation 1345 (May 2000))

5 ADAMTS proteins and ADAMTS protein stimulators and inhibitors have important therapeutic uses, including treatment of arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ  
10 transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head  
15 trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility diabetic shock and other diseases  
20 characterized by metalloproteinase activity and/or characterized by mammalian adamalysin activity.

#### Summary Of The Invention

This invention relates to a novel ADAMTS protein, designated ADAMTS-E and a shorter splice variant thereof called ADAMTS-E2, and to related polynucleotides and  
25 polypeptides. The invention also relates to production of the proteins and polypeptides and to related assays. The invention further relates to methods for identifying substrates of the proteins, for identifying inhibitors or stimulators of the proteins, and to the use of the polypeptides or polynucleotides of the invention in diagnostic, biotherapeutic, and gene therapy methods.

30 In particular, the invention relates to an isolated polynucleotide molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence having at least 80% identity to a nucleotide sequence encoding ADAMTS-E polypeptide of SEQ ID NO: 2, or the metalloproteinase, disintegrin domain, prodomain, or thrombospondin (TSP) motif thereof;
- 35 (b) a nucleotide sequence having at least 80% identity to a nucleotide sequence encoding ADAMTS-E2 polypeptide of SEQ ID NO: 8, or the metalloproteinase, disintegrin domain, or prodomain thereof;

(c) a nucleotide sequence of at least 15 contiguous nucleotides that hybridizes under stringent conditions to the polynucleotide molecule of SEQ ID NO: 1 or to the polynucleotide of SEQ ID NO: 7; and

(d) the complement of the nucleotide sequence of (a) or (b) or (c).

5        Such an isolated polynucleotide molecule, can, for example, comprise DNA or RNA.

      In one embodiment, the nucleotide sequence is at least 80% identical to SEQ ID NO: 1 or the metalloproteinase or disintegrin domain, or prodomain, encoding sequences thereof. In another embodiment the nucleotide sequence is the ADAMTS-E polypeptide encoding sequence of SEQ ID NO: 1 or the metalloproteinase or disintegrin domain, or prodomain, or TSP motif, encoding sequences thereof. In another aspect, the isolated polynucleotide molecule comprises the mouse orthologue of the polynucleotide molecule of SEQ ID NO: 1, or the metalloproteinase or disintegrin domain, or prodomain, or TSP motif thereof, the mouse orthologue preferably having the sequence of SEQ ID NO. 3.

10

      In another embodiment, the nucleotide sequence is at least 80% identical to SEQ ID NO: 7 or the metalloproteinase or disintegrin domain, or prodomain, encoding sequences thereof. In another embodiment the nucleotide sequence is the ADAMTS-E2 polypeptide encoding sequence of SEQ ID NO: 7 or the metalloproteinase or disintegrin domain, or prodomain encoding sequences thereof. In another aspect, the isolated polynucleotide molecule comprises the mouse orthologue of the polynucleotide molecule of SEQ ID NO: 7, or the metalloproteinase or disintegrin domain, or prodomain, thereof.

15

20

      In a further embodiment, the invention relates to a polypeptide encoded by the isolated polynucleotide molecules of the invention. For example, the polypeptide of the invention can comprise an amino acid sequence which is at least 80% identical to SEQ ID NO: 2 or the metalloproteinase or disintegrin domains, or prodomain, or TSP motif thereof, or at least 80% identical to SEQ ID NO: 8 or the metalloproteinase or disintegrin domains, or prodomain, thereof, or an amino acid sequence of at least about 10 contiguous amino acids of ADAMTS-E or of ADAMTS-E2. The polypeptide of the invention can also comprise an amino acid sequence which is at least 80% identical to SEQ ID NO: 9. In a preferred embodiment, the polypeptide comprises SEQ ID NO: 2 or the metalloproteinase or disintegrin domains, prodomain or a TSP motif, thereof, or SEQ ID NO: 8 or the metalloproteinase or disintegrin domains or prodomain thereof. In an especially preferred embodiment, the polypeptide comprises the metalloproteinase domain. In another aspect, the polypeptide comprises the mouse orthologue of the polypeptide of SEQ ID NO: 2, or the metalloproteinase or disintegrin domain, or prodomain, or TSP motif thereof, the mouse orthologue preferably having the sequence of SEQ ID NO. 4.

25

30

35

      In another aspect, the invention relates to an expression system comprising an isolated polynucleotide molecule of the invention. In one embodiment, the expression system

is capable of producing an ADAMTS-E polypeptide that comprises an amino acid sequence that has at least 80% identity with a polypeptide of SEQ ID NO: 2 or the metalloproteinase or disintegrin domains, or prodomain, or TSP motif thereof, when said expression system is present in a compatible host cell. In one embodiment of this aspect of the invention, the expression system is capable of producing an ADAMTS-E polypeptide encoded by a polynucleotide of the invention. In a further aspect, the invention relates to an expression system capable of producing an ADAMTS-E2 polypeptide that comprises an amino acid sequence that has at least 80% identity with a polypeptide of SEQ ID NO: 8 or the metalloproteinase or disintegrin domains or prodomain thereof, when said expression system is present in a compatible host cell. In another embodiment of the invention, the expression system is capable of producing an ADAMTS-E2 polypeptide encoded by a polynucleotide of the invention.

In another aspect, the invention relates to a host cell that comprises the expression system of the invention.

In another aspect, the invention relates to a process for producing an ADAMTS-E polypeptide or an ADAMTS-E2 polypeptide that comprises culturing a host cell of the invention under conditions sufficient for production of the polypeptide, and recovering the polypeptide from cell culture.

In another aspect, the invention relates to a process for producing a cell which produces an ADAMTS-E polypeptide or an ADAMTS-E2 polypeptide comprising transforming or transfecting a host cell with an expression system of the invention such that the host cell, under appropriate culture conditions produces the ADAMTS-E polypeptide or the ADAMTS-E2 polypeptide as the case may be.

In another aspect, the invention relates to an antibody that is immunospecific for an ADAMTS-E polypeptide of the invention or the ADAMTS-E2 polypeptide of the invention. The invention also relates to inhibitors, stimulators, and substrates of the polypeptide of the invention.

In a further aspect, the invention relates to a method for treating a subject in need of altering activity or expression of ADAMTS-E or of ADAMTS-E2 comprising administering to the subject a therapeutically effective amount of a stimulator or inhibitor of ADAMTS-E or of ADAMTS-E2.

In another aspect, the invention relates to a method for treating a subject in need of altering activity or expression of ADAMTS-E or of ADAMTS-E2 comprising administering to the subject a polynucleotide of the invention in order to alter said activity or expression. The invention also relates to a method for treating a subject in need of altering activity or expression of ADAMTS-E or of ADAMTS-E2 comprising administering to the subject a

therapeutically effective amount of a polypeptide that competes with ADAMTS-E or ADAMTS-E2 for its ligand, substrate, or receptor.

5 The invention also relates to a process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of ADAMTS-E or ADAMTS-E2 in a subject comprising determining presence or absence of a mutation in a nucleotide sequence encoding ADAMTS-E or ADAMTS-E2 in the genome of the subject. Alternately, the invention relates to a process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of ADAMTS-E or ADAMTS-E2 in a subject comprising analyzing for presence or amount of ADAMTS-E or ADAMTS-E2 expression in a sample  
10 derived from the subject.

In another aspect, the invention relates to a method for identifying compounds which stimulate, inhibit, or bind to ADAMTS-E or ADAMTS-E2, comprising:

- 15 (a) contacting a candidate compound with cells expressing a polypeptide of the invention, or with cell membranes from cells expressing the polypeptide, or the media conditioned by cells expressing the polypeptide, or a purified composition of the polypeptide; and
- (b) determining inhibition or stimulation of an ADAMTS-E or ADAMTS-E2 activity, or binding of the candidate compound to the polypeptide.

The invention also relates to a method for detecting a polynucleotide encoding  
20 ADAMTS-E or ADAMTS-E2 in a biological sample containing nucleic acid material comprising:

- (a) hybridizing an isolated polynucleotide of the invention that is specific to ADAMTS-E or ADAMTS-E2 to the nucleic acid material of the biological sample, thereby forming a hybridization complex; and
- 25 (b) detecting the hybridization complex, wherein presence of the hybridization complex correlates with the presence of the polynucleotide encoding ADAMTS-E or ADAMTS-E2 in the biological sample.

In a further embodiment, the invention relates to a method for identifying a substrate for ADAMTS-E or ADAMTS-E2 comprising contacting a polypeptide comprising an  
30 enzymatically active polypeptide of the invention with a candidate substrate and determining either conversion of substrate to product or binding of the polypeptide to the substrate.

The invention also relates to a method for treating arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's  
35 disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease,

epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility or diabetic shock comprising administering a therapeutically effective amount of a stimulator or inhibitor of ADAMTS-E in combination with a pharmaceutically acceptable carrier.

The invention also relates to a method for treating arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility or diabetic shock comprising administering a polypeptide of the invention in combination with a pharmaceutically acceptable carrier.

The invention also relates to a method for treating arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression,

peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility or diabetic shock comprising administering a polynucleotide of the invention in combination with a pharmaceutically acceptable carrier.

The invention further relates to a pharmaceutical composition for the treatment of arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility or diabetic shock comprising a therapeutically effective amount of a stimulator or inhibitor, of ADAMTS-E or ADAMTS-E2 in combination with a pharmaceutically acceptable carrier.

The invention also relates to a pharmaceutical composition for the treatment of arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility or diabetic shock comprising a polypeptide of the invention in combination with a pharmaceutically acceptable carrier.

The invention also relates to a pharmaceutical composition for the treatment of arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility or diabetic shock comprising a polynucleotide of the invention in combination with a pharmaceutically acceptable carrier.

#### Brief Description Of The Drawings

FIG. 1 shows the polynucleotide sequence of ADAMTS-E [SEQ ID NO:1].

FIG. 2 shows the polypeptide sequence of ADAMTS-E [SEQ ID NO:2].

FIG. 3 shows domains of the ADAMTS family of proteins, and the sequences that correspond to those domains within the partial ADAMTS-E polypeptide.

FIG. 4 shows homology within the metalloproteinase domain of the ADAMTS-E polypeptide sequence with those of other ADAMTS proteins.

FIG. 5 shows the results of a semi-quantitative PCR analysis of ADAMTS-E expression and regulation by proinflammatory treatments in chondrocytes prepared from osteoarthritic cartilage.

FIG. 6 shows an alignment of human ADAMTS-E with the homolog predicted from a mouse genomic sequence.

SEQ ID NO: 1 shows the polynucleotide sequence of human ADAMTS-E.

SEQ ID NO: 2 shows the polypeptide sequence of ADAMTS-E.

SEQ ID NO: 3 shows the polynucleotide sequence of the mouse orthologue of ADAMTS-E.

SEQ ID NO: 4 shows the polypeptide sequence of mouse ADAMTS-E.

SEQ ID NOS: 5 and 6 show primer sequences designed from the polynucleotide sequence encoding ADAMTS-E.

SEQ ID NO: 7 shows the polynucleotide sequence of human ADAMTS-E2.

SEQ ID NO: 8 shows the polypeptide sequence of ADAMTS-E2.



SEQ ID NO: 9 shows an N-terminally extended polypeptide sequence of ADAMTS-E2.

#### Detailed Description Of The Invention

5 We have found relatively high levels of polynucleotide encoding the ADAMTS-E/ADAMTS-E2 protein in cDNA prepared from the chondrocytes of osteoarthritic cartilage as well as in cDNA libraries derived from human brain, heart, lung liver, kidney and spleen.

#### **Definitions**

The following definitions are provided to facilitate understanding of terms used herein.

10 "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example tritylated bases and unusual bases such as inosine. A variety of modifications have been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres.

30 "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. "Polypeptides" may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same

type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, *Proteins - structure and molecular properties*, 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York, 1993; F. Wold, *Posttranslational protein modifications: perspectives and prospects*, pgs. 1-12 in *Posttranslational covalent modification of proteins*, B.C. Johnson, Ed., Academic Press, New York, 1983; S. Seifter and S. Englund, *Analysis for protein modifications and nonprotein cofactors*, 182 *Methods of Enzymology* 626 (1990); S.I. Rattan et al., *Protein synthesis, posttranslational modifications, and aging*, 663 *Ann NY Acad Sci* 48 (1992).

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions and/or deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or a polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is

obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, for example, *Computational molecular biology*, A.M. Lesk, ed., Oxford University Press, New York, 1988; *Biocomputing: informatics and genome projects*, D.W. Smith, ed., Academic Press, New York, 1993; *Computer analysis of sequence data, part 1*, A.M. Griffin, and H.G. Griffin, eds., Humana Press, New Jersey, 1994; *Sequence analysis in molecular biology*, G. von Heinje, ed., Academic Press, 1987; and *Sequence analysis primer*, M. Gribskov and J. Devereux, eds., M Stockton Press, New York, 1991. While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package; J. Devereux, et al., *A comprehensive set of sequence analysis programs for the VAX*, 12(1) Nucleic Acids Research 387 (January 1984); BLASTP; BLASTN; FASTA; S.F. Altschul et al., *Basic local alignment search tool*, 215(3) Journal of Molecular Biology 403 (October 1990). Among the methods stated above to determine identity, the preferred method is BLASTP.

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of FIG. 1, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of FIG. 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of FIG. 2, it is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of FIG. 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the

reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

#### **ADAMTS-E Polypeptides**

In one aspect, the present invention relates to ADAMTS-E polypeptides. The ADAMTS-E polypeptides include the polypeptide of FIG. 2, as well as polypeptides comprising an amino acid sequence of FIG. 2, and polypeptides comprising an amino acid sequence that has at least 80% identity to that of FIG. 2, preferably at least 90% identity, more preferably at least 95% identity to FIG. 2, and most preferably at least 97-99% identity to FIG. 2. The invention also includes the polypeptide of SEQ ID NO: 8, another specific example of an ADAMTS-E polypeptide herein referred to as ADAMTS-E2, as well as polypeptides comprising an amino acid sequence of SEQ ID NO: 8, and polypeptides comprising an amino acid sequence that has at least 80% identity to that of SEQ ID NO: 8, preferably at least 90% identity, more preferably at least 95% identity to SEQ ID NO: 8, and most preferably at least 97-99% identity to SEQ ID NO: 8.

The ADAMTS-E polypeptides may be in the form of an unprocessed or partially processed precursor, or the "mature" protein, which may in turn be a part of a larger protein such as a fusion protein. The mature form should normally begin with or near amino acid 67 and continue to the carboxyl terminus. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification or identification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the ADAMTS-E polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned ADAMTS-E polypeptides. As with ADAMTS-E polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of ADAMTS-E polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic

regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Also preferred are biologically active fragments. Biologically active fragments are those that mediate one or more ADAMTS-E activities, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Most preferred are fragments that comprise one or more of the domains or motifs shown in Fig. 3. In particularly preferred embodiments, the fragment comprises the metalloproteinase domain or the disintegrin domain. In another embodiment, the polypeptide comprises amino acids 392-420 of SEQ ID NO: 2, which encompasses an extension of the zinc binding motif.

Such fragments are conventionally employed by themselves, or as part of fusion proteins. For example, expression vectors can be constructed that will express a fusion protein comprising a protein or polypeptide of the present invention. Such fusion proteins can be used, *e.g.*, to raise antisera against the protein, to study the biochemical properties of the protein, to engineer a protein exhibiting different immunological or functional properties, to aid in the identification or purification, to improve the stability, of a recombinantly-expressed protein, or as therapeutic agents. Possible fusion protein expression vectors include but are not limited to vectors incorporating sequences that encode  $\beta$ -galactosidase and *trpE* fusions, maltose-binding protein fusions (pMal series; New England Biolabs), glutathione-S-transferase fusions (pGEX series; Pharmacia), polyhistidine fusions (pET series; Novagen Inc., Madison, WI), and thioredoxin fusions (pTrxFus; Invitrogen, Carlsbad, CA). As one example, the disintegrin domain or TSP domain, or a polypeptide comprising a variant or fragment thereof, may be administered alone, or as part of a fusion protein, to competitively inhibit in vivo or in vitro interactions with the native disintegrin domain or TSP domain. Methods are well-known in the art for constructing expression vectors encoding these and other fusion proteins.

Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions, *i.e.*, those that substitute a residue with another of like characteristics. Typical conservative substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg; and among the aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5 to 10, 1 to 5, or 1 to 2 amino acids are substituted, deleted, or added in any combination.

The ADAMTS-E polypeptides of the invention can be prepared in any suitable manner. The polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, and polypeptides produced by a combination of these methods. These methods are well understood in the art.

Another embodiment of the present invention is an isolated ADAMTS-E polypeptide, a further embodiment of the invention is an isolated ADAMTS-E2 polypeptide. An isolated polypeptide is one that has been substantially removed from its natural milieu. An isolated ADAMTS-E polypeptide or an isolated ADAMTS-E2 polypeptide can, for example, be obtained from its natural source, be produced using recombinant technology, or be synthesized chemically. An isolated ADAMTS-E polypeptide/ADAMTS-E2 polypeptide can be full-length ADAMTS-E polypeptide/ADAMTS-E2 polypeptide, the predicted mature form processed by furin cleavage of the prodomain (amino acid 67 with the predicted mature form beginning GTGA...), or any homologue of such a polypeptide, such as an ADAMTS-E or ADAMTS-E2 polypeptide in which amino acids have been deleted, inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycosylphosphatidyl inositol). A homologue of an ADAMTS-E polypeptide is a polypeptide having an amino acid sequence that is sufficiently similar to a natural ADAMTS-E polypeptide amino acid sequence that a nucleic acid sequence encoding the homologue is capable of hybridizing under stringent conditions to a nucleic acid sequence encoding the natural ADAMTS-E polypeptide amino acid sequence disclosed herein. As used herein, "stringent hybridization conditions" refers to hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% SDS, 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (see Ausubel *et al.* (eds.), 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). A homologue of ADAMTS-E polypeptide also includes a polypeptide having an amino acid sequence that is sufficiently cross-reactive such that the homologue has the ability to elicit an immune response against at least one epitope of naturally-occurring ADAMTS-E polypeptide. Preferably the homologue retains one or more biological activities of ADAMTS-E.

The minimal length of a protein homologue of the present invention is sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. As such, the size of the nucleic acid molecule encoding such a protein homologue is dependent on nucleic acid composition, percent homology between the nucleic acid molecule and complementary sequence, as well as upon hybridization conditions per se (e.g., temperature, salt concentration and formamide concentration). The minimal size of such nucleic acid is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecules are GC-rich and at least about 15 to about 17 bases in length if they are AT-rich.

The minimal size of a nucleic acid molecule used to encode an ADAMTS-E protein homologue of the present invention is from about 20 to about 25 nucleotides in length. There is no limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in

that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof. In one embodiment, the minimal size of an ADAMTS-E protein homologue of the present invention is from 10, more preferably 12, even more preferably 25, amino acids in length. In another embodiment, a polypeptide of the invention comprises an amino acid sequence of more than about 10, or 25, preferably more than 75, more preferably more than 100, amino acids that is identical to an amino acid sequence of SEQ ID NO: 2 or to an amino acid sequence of SEQ ID NO: 8. Preferred protein or polypeptide sizes depend on whether a full-length, multivalent protein (i.e., fusion protein having more than one domain each of which has a function), or a functional portion of such a protein is desired. Functional portions are obtainable based on the domains described herein, knowledge in the art concerning such domains and known assays for such domain, or its functional activity. Useful protein fragments or other polypeptides can also be screened for based on antigenic cross-reactivity with the ADAMTS-E protein of SEQ ID NO: 2 or the ADAMTS-E2 protein of SEQ ID NO: 8..

ADAMTS-E protein homologues of the invention include allelic variations of the natural gene encoding the ADAMTS-E proteins. A "natural" gene is that found most often in nature. ADAMTS-E protein homologues can be produced using techniques known in the art, including, but not limited to, direct modifications to a gene encoding a protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

The present invention, encompasses the ADAMTS-E proteins that have undergone posttranslational modification. Such modification can include, for example, glycosylation (e.g., including addition of N-linked and/or O-linked oligosaccharides) or posttranslational conformational changes or posttranslational deletions.

Based on the 31-59% identity in the metalloprotease domain of ADAMTS-E as compared to other ADAMTS family members, ADAMTS-E may have one or more proteolytic activities (e.g. collagenase, aggrecanase, procollagen protease) as well as anti-angiogenic activities that may or may not require the presence of the thrombospondin domains. See, FIGS. 3 and 4. These possible activities of ADAMTS-E can be tested using techniques known to those skilled in the art. See, e.g., P.D. Brown et al., *Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines*, 50(19) Cancer Research 6184 (October 1990); F. Vazquez et al., *METH-1 a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity*, 274 The Journal of Biological Chemistry 23349 (Aug. 1999); E.C. Arner et al., *Generation and characterization of aggrecanase*, 274 The Journal of Biological Chemistry 6594 (Mar. 1999); A. Colige et al., *cDNA cloning and expression of bovine procollagen I N-proteinase: A new member of the superfamily of zinc-*

*metalloproteinases with binding sites for cell and other matrix components* 94 Proceedings of the National Academy of Sciences (USA) 2374 (March 1997).

#### **ADAMTS-E Polynucleotides**

Another aspect of the invention relates to ADAMTS-E polynucleotides. ADAMTS-E  
5 polynucleotides include isolated polynucleotides which encode the ADAMTS-E polypeptides  
and fragments, and polynucleotides closely related thereto. More specifically, ADAMTS-E  
polynucleotides of the invention include a polynucleotide comprising the nucleotide sequence  
set forth in FIG. 1 encoding a ADAMTS-E polypeptide of FIG. 2, as well as a polynucleotide  
10 comprising the nucleotide sequence of SEQ ID NO: 7 encoding a ADAMTS-E2 polypeptide of  
SEQ ID NO: 8. Another aspect of the invention is a polynucleotide having the particular  
sequence of FIG. 1, and a polynucleotide having the particular sequence of SEQ ID NO: 7.  
ADAMTS-E polynucleotides further include a polynucleotide comprising a nucleotide  
sequence that has at least 80% identity to a nucleotide sequence encoding the ADAMTS-E  
15 polypeptide of FIG. 2 and a polynucleotide comprising a nucleotide sequence that has at least  
80% identity to a nucleotide sequence encoding the ADAMTS-E2 polypeptide of SEQ ID NO:  
8. Also included in the invention is a polynucleotide that is at least 80% identical to the  
polynucleotide sequence of FIG. 1, and a polynucleotide that is at least 80% identical to the  
polynucleotide of SEQ ID NO: 7. In this regard, polynucleotides at least 90% identical are  
20 particularly preferred, and those with at least 95% are especially preferred. Furthermore,  
those with at least 97% are highly preferred and those with at least 98-99% are most highly  
preferred, with at least 99% being the most preferred.

In one embodiment, the nucleic acid molecule of the invention has a nucleotide  
sequence has between 1 and 50, more preferably between 1 and 25, and most preferably  
between 1 and 5 nucleotides inserted, deleted, or substituted with respect to the sequence of  
25 SEQ ID NO: 1. In another embodiment, the nucleic acid molecule of the invention has a  
nucleotide sequence between 1 and 50, more preferably between 1 and 25, and most  
preferably between 1 and 5 nucleotides inserted, deleted, or substituted with respect to the  
sequence of SEQ ID NO: 7.

ADAMTS-E polynucleotides of the invention also encompass nucleotide sequences  
30 which have sufficient identity to the nucleotide sequence contained in FIG. 1 or to the  
nucleotide sequence of SEQ ID NO: 7, to hybridize under conditions useable for amplification  
or for use as a probe or marker for ADAMTS-E. Such sequences are typically 15 to 25  
nucleotides in length with a target of 50% GC content and useful in PCR amplification or  
oligonucleotide hybridization methods well known to those skilled in the art. (See, e.g.,  
35 Promega Protocols and Applications Guide, Third Edition, (1996), ISBN 1-8822474-57-1).

In one embodiment, the isolated nucleic acid molecule comprises a fragment of SEQ  
ID NO: 1 that is specific for ADAMTS-E, i.e., specifically acts as a probe for SEQ ID NO: 1.



The fragment may be at least, e.g., 15, 25, 35, 45 or 75 nucleotides in length. In another embodiment, the isolated nucleic acid molecule comprises a fragment of SEQ ID NO: 7 that is specific for ADAMTS-E2, i.e. specifically acts as a probe for SEQ ID NO: 7; this fragment will be derived from residues 1556 to 1774, and may be at least, e.g. 15, 25, 35, 45, or 75 nucleotides in length.

Another embodiment of the present invention is an isolated nucleic acid molecule capable of hybridizing, under stringent conditions, with ADAMTS-E polypeptide cDNA (FIG. 1 or SEQ ID NO: 7) encoding an ADAMTS-E protein of the present invention.

An isolated nucleic acid of the invention can include DNA, RNA or derivatives of either DNA or RNA.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (i.e., complete) gene or a portion thereof capable of forming a stable hybrid with that gene. As used herein, the phrase "at least a portion of" an entity refers to an amount of the entity that is at least sufficient to have functional aspects of that entity. For example, at least a portion of a nucleic acid sequence, as used herein, is an amount of a nucleic acid sequence capable of forming a stable hybrid with a particular desired gene (e.g., ADAMTS genes) under stringent hybridization conditions. An isolated nucleic acid molecule of the present invention can also be produced using recombinant technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated ADAMTS-E protein nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in a manner that does not substantially interfere with the nucleic acid molecule's ability to encode an ADAMTS-E protein of the present invention or to form stable hybrids under stringent conditions with natural nucleic acid molecule isolates encoding an ADAMTS-E protein.

The invention also provides polynucleotides that are complementary to ADAMTS-E polynucleotides described above.

#### **Expression of ADAMTS-E**

In one embodiment, an isolated ADAMTS-E protein of the present invention is produced by culturing a recombinant cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. Preferred cells include bacterial (e.g., *E. coli*), yeast (e.g., *Pichia*), insect (e.g., SF9) or mammalian cells (e.g., CHO, Cos 7, and HEK 293). The recombinant cell is capable of expressing the ADAMTS-E protein and is produced by transforming a host cell with one or more nucleic acid molecules of the present invention. Such recombinant cells are part of the present invention. Suitable transformation techniques include, but are not limited to, transfection, electroporation, microinjection,

lipofection, adsorption and protoplast fusion. Recombinant cells of the invention may remain unicellular or may grow into a tissue organ or a multicellular organism. Nucleic acid molecules of the present invention used to transform cells according to conventional techniques can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained.

Suitable host cells for transforming a cell include any cell capable of producing ADAMTS-E proteins of the present invention after being transformed with at least one nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule the present invention. Suitable host cells include bacterial, fungal (including yeast), insect, animal and plant cells.

The present invention also encompasses a recombinant vector which comprises a polynucleotide of the present invention inserted into a vector capable of delivering the polynucleotide into a host cell. Such a vector normally contains heterologous nucleic acid sequences, for example nucleic acid sequences that are not naturally found adjacent to ADAMTS-E protein nucleic acid molecules of the present invention. The vector can be either DNA or RNA, and either prokaryotic or eukaryotic, and is typically a virus or a plasmid. Recombinant vectors can be used in cloning, sequencing, and/or otherwise manipulating or expressing ADAMTS-E polynucleotides of the present invention.

In one embodiment of the invention, a recombinant cell is produced by transforming a host cell with one or more recombinant molecules, each comprising one or more polynucleotide molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences. The phrase "operatively linked" refers to a nucleic acid molecule inserted into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, the phrase "expression vector" refers to a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule.

Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include vectors that effect direct gene expression in bacterial, fungal, insect, animal, and/or plant cells. Nucleic acid molecules of the present invention can be operatively linked to expression vectors containing regulatory sequences such as promoters, operators, repressors, enhancers, termination sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules. Transcription control sequences that can be used in the present invention include those capable of controlling the initiation, elongation, and termination of transcription. Particularly important

transcription control sequences are those which control transcriptional initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include those that function in one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art.

- 5 Preferred transcription control sequences include those which function in bacterial yeast and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, *oxy-pro*, *omp/lpp*, *rmB*, bacteriophage lambda ( $\lambda$ ) (such as  $\lambda_P$  and  $\lambda P_R$  and fusions that include such promoters), bacteriophage T7, T7lac, bacteriophage T3, bacteriophage SP6, bacteriophage SPO1, metallothionein, alpha mating factor, baculovirus, vaccinia virus, herpesvirus, poxvirus, adenovirus, simian virus 40, retrovirus action, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences, as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by
- 10 interferons or interleukins). Transcription control sequences useful in practicing the present invention include naturally occurring sequences associated with DNA encoding an ADAMTS-E protein.

- Preferred nucleic acid molecules for insertion into an expression vector include nucleic acid molecules encoding at least a portion of an ADAMTS-E protein, or a homologue thereof. Expression vectors of the present invention may also contain fusion sequences, e.g., as discussed above, which allow expression of nucleic acid molecules of the present invention as fusion proteins. Inclusion of a fusion sequence in an ADAMTS-E nucleic acid molecule of the present invention can enhance stability during production, storage, or use of the protein encoded by the nucleic acid molecule. Furthermore, a fusion segment can simplify purification of an ADAMTS-E protein, enabling purification by affinity chromatography. Fusion segments can be of any size that affords the desired function (e.g., increased stability and/or easier purification). It is within the scope of the present invention to use one or more fusion segments. Fusion segments can be joined to amino and/or carboxyl termini of the ADAMTS-E protein or polypeptide of the present invention. Linkages between fusion
- 20 segments and ADAMTS-E proteins can be constructed to be susceptible to cleavage to enable straightforward recovery of the ADAMTS-E proteins. Fusion proteins are preferably produced by culturing a recombinant cell transformed with nucleic acid sequences that encode the fusion segment attached to either the carboxyl and/or amino terminal end of a ADAMTS-E polypeptide of the invention.

- 35 The present invention includes recombinant cells resulting from transformation with a nucleic acid molecule of the present invention. Preferred recombinant cells are transformed with a nucleic acid molecule that encodes at least a portion of an ADAMTS-E protein, or a

homologue thereof. Amplifying the copy number of nucleic acid sequences of the invention can be accomplished by increasing the copy number of the nucleic acid sequence in the cell's genome or by introducing additional copies of the nucleic acid sequence by transformation. Copy number amplification is conducted in a manner such that greater amounts of enzyme  
5 are produced, leading to enhanced conversion of substrate to product. Transformation can be accomplished using any process by which nucleic acids are transformed into cells to enhance enzyme synthesis. Prior to transformation, the nucleic acid sequence can, if desired, be manipulated to encode an enzyme having a higher specific activity.

In accordance with the present invention, recombinant cells are used to produce an  
10 ADAMTS-E protein of the present invention by culturing such cells under conditions effective to produce such a protein, and the protein recovered. Effective conditions include, but are not limited to, appropriate media, bioreactor, temperature, pH and oxygen conditions that permit protein production. Suitable media are typically aqueous and comprise assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals  
15 and other nutrients, such as vitamins. The medium may comprise complex nutrients, or may be minimal.

Cells of the present invention can be cultured in conventional fermentation bioreactors, which include, but are not limited to, batch, fed-batch, cell recycle, and continuous fermentors. Culturing can also be conducted in shake flasks, test tubes, microtiter  
20 dishes, and petri plates. Culturing is carried out at a temperature, pH and oxygen content appropriate for the recombinant cell. Such culturing is within the expertise of one of ordinary skill in this art.

Depending on the vector and host system used for production, resultant ADAMTS-E proteins may either remain within the recombinant cell or be secreted into the fermentation medium. "Recovering the protein" according to the invention may involve simply collecting  
25 the fermentation medium or cells containing the protein and need not include additional steps of separation or purification. ADAMTS-E proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic  
30 interaction chromatography, gel filtration chromatography, reverse phase chromatography, chromatofocusing and differential solubilization.

In addition, an ADAMTS-E protein of the present invention can be produced by isolating the ADAMTS-E protein from cells expressing the ADAMTS-E protein recovered from transgenic animal, or from fluid, such as milk, recovered from such an animal. An isolated  
35 protein or polypeptide of the present invention can be used to formulate a therapeutic composition as discussed further below.

### **Antibodies to ADAMTS-E**

The present invention also includes antibodies capable of selectively binding to an ADAMTS-E protein or polypeptide of the present invention. Polyclonal populations of anti-ADAMTS-E antibodies can be contained in an ADAMTS-E antiserum. Binding can be measured using a variety of methods known to those skilled in the art including immunoblot assays, immunoprecipitation assays, enzyme immunoassays (e.g., ELISA), radioimmunoassay, immunofluorescent antibody assays and immunoelectron microscopy; see, for example, Sambrook et al., *Molecular cloning: a laboratory manual*, Cold Spring Harbor Labs Press, 1989.

Antibodies of the present invention can be either monoclonal or polyclonal antibodies and can be prepared using techniques standard in the art. Antibodies of the present invention include functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies that are capable of selectively binding to at least one of the epitopes of the protein used to obtain antibodies. Preferably, antibodies are raised in response to proteins that are encoded, at least in part, by an ADAMTS-E nucleic acid molecule.

### **Identification of ADAMTS-E Substrates**

The present invention also encompasses methods for identifying ADAMTS-E substrates. Such methods include those wherein a candidate substrate is contacted with a polypeptide comprising an enzymatically active ADAMTS-E polypeptide of the invention, and conversion of substrate to product is determined, or binding of polypeptide to the candidate substrate determined. The invention also encompasses rational drug design conducted using computer software that calculates interactions between candidate compounds and polypeptides or polynucleotides of the invention.

Substrates may be identified by a candidate protein or synthetic substrate approach. For example, candidate proteins can be cast within an agarose gel matrix and the ability of the ADAMTS-E protein to digest the protein determined using protein zymography. See, P.D. Brown et al., *Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines*, 50(19) Cancer Research 6184 (October 1990). Alternatively, a phage display or fluorometric peptide library can be screened to identify substrates of the protein. See, D.R. O'Boyle et al., *Identification of a novel peptide substrate of HSV-1 protease using substrate phage display*, 236(2) Virology 338 (September 1997).

### **Stimulators/inhibitors of ADAMTS-E**

The present invention also includes assays for determining stimulators and/or inhibitors of ADAMTS-E. Assays for determining aggrecanase, collagenase, procollagen protease and/or angiogenic activities may be used to identify stimulator or inhibitor

compounds, preferably small molecular weight compounds of less than 700 daltons. The compounds may contain a hydroxamic acid moiety or an optionally substituted heterocyclic nucleus, or an aryl or heteroaryl sulfonamide moiety, which compounds inhibit or stimulate the activity of endogenous or recombinant ADAMTS-E. E.C. Arner et al., *Generation and characterization of aggrecanase. A soluble, cartilage-derived aggrecan-degrading activity*, 274 Journal of Biological Chemistry 6594 (March 1999); M.D. Tortorella et al., *supra*; A. Colige et al., *supra*; K. Kuno et al., *supra*. ELISA or fluorescent substrate assays can be performed to determine stimulators or inhibitors, or to determine specific proteolytic activity, of an ADAMTS-E protein.

#### Diagnostic Assays

The present invention also includes processes for diagnosing diseases or susceptibility to diseases related to expression and/or activity of ADAMTS-E polypeptides. Such diseases may be identified by determining the presence or absence of a mutation in the nucleotide sequence encoding said ADAMTS-E polypeptides in the genome of a patient. Alternately, the presence or amount of ADAMTS-E polypeptides in a sample derived from a patient may be determined as an indicator of disease or susceptibility to disease. Such diagnosis may be performed for diseases including the following: arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility, diabetic shock and other diseases characterized by metalloproteinase activity and/or characterized by mammalian adamalysin activity.

#### Therapeutic compositions and uses of ADAMTS-E

In one embodiment of the present invention, an antibody, stimulator, inhibitor, substrate and/or variant of an ADAMTS-E, or a polypeptide or polynucleotide of the invention, is employed in a therapeutic composition for treatment of arthritis (osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, acute

respiratory distress syndrome, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity and rejection, cachexia, allergy, cancer (such as solid tumor cancer including colon, breast, lung, prostate, brain, and hematopoietic malignancies including leukemia and lymphoma), tissue ulcerations, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joints implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neurodegenerative diseases (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, infertility, diabetic shock or other diseases characterized by metalloproteinase activity and/or mammalian adamalysin activity.

In one embodiment, polynucleotides of the invention can, for example, be employed to transform cells in gene therapy application, e.g., as part of in vivo or ex vivo gene therapy. Polynucleotides can also be employed in antisense therapy, and in the construction of ribozymes. Use of polynucleotides in these methods is known to those skilled in this art.

For administration to mammals, including humans, a variety of conventional routes may be used including oral, parenteral (e.g., intravenous, intramuscular or subcutaneous), buccal, anal and topical.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelation and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof. In the case of animals, they are advantageously contained in an animal feed or drinking water in a concentration of 5-5000 ppm, preferably 25 to 500 ppm.

For parenteral administration (intramuscular, intraperitoneal, subcutaneous and intravenous use) a sterile injectable solution of the active ingredient is usually prepared.

Solutions of the therapeutic compound in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH of greater than 8, if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable for intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. In the case of animals, compounds can be administered intramuscularly or subcutaneously at dosage levels of about 0.1 to 50 mg/kg/day, advantageously 0.2 to 10 mg/kg/day given in a single dose or up to 3 divided doses.

Active compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, active compounds are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

A therapeutic composition of the present invention can be administered to any subject having a medical disorder as herein described. Acceptable protocols by which to administer therapeutic compounds of the present invention in an effective manner vary according to individual dose size, number of doses, frequency of dose administration and mode of administration. Determination of appropriate protocols is accomplished by those skilled in the art without undue experimentation. An effective dose refers to a dose capable of treating a subject for a medical disorder as described herein. Effective doses vary depending upon, for example, the therapeutic composition used, the medical disorder being treated and the size and type of recipient animal.

The dosage and length of treatment depends on the disease state being treated. The duration of treatment may be a day, a week or longer and may last over the lifetime of the patient.



The present invention is illustrated by the following Examples, which are intended to exemplify, and not limit, the invention.

#### Example 1

##### **Identification of ADAMTS-E**

5 For identification of novel ADAMTS gene family members, a non-redundant set of publicly available protein sequences was assembled (accession numbers: D67076, AJ003125, AB002364, AB014588). A series of low stringency BLAST searches were then performed against the LifeSeq Gold™ database (Incyte) using each of the above protein sequences as queries. A 285bp EST was identified in the Incyte database containing  
10 metalloproteinase and disintegrin domains. This sequence was used to design PCR primers, and a panel of libraries was screened to determine a source for further cloning. Expression was found in a human brain cDNA library, which was screened using GeneTrapper homology capture. A clone (#10) was obtained which yielded a sequence encoding a prodomain, furin-cleavage site, and complete metalloproteinase and disintegrin domains. This sequence was  
15 used to search the Incyte database for more information. An overlapping Incyte clone (#2) from a human heart library was identified that added a TSP1 motif and four TSP1-like submotifs to the clone #10 sequence. Finally, an overlapping clone (#11) was obtained by 3' RACE from human spleen RNA that contained an in-frame stop codon and polyadenylation signal. The composite nucleotide (FIG. 1) and amino acid sequences (FIG. 2) are shown as  
20 well as the domain structure (FIG. 3). BLASTP 2.0.9 analysis showed high homology to a number of ADAMTS family members, particularly ADAMTS-6 (FIG 4).

A BLAST analysis of the human ADAMTS-E sequence against the high throughput genomic (HTG) section of Genbank revealed a close match to a mouse genomic contig (approximately 85-90% identity at the nucleotide level for each exon match) indicating this  
25 was the mouse orthologue of ADAMTS-E. The GENSCAN gene prediction program was run on this sequence and the predicted protein was compared to the human sequence (FIG. 6). Most of the mouse sequence appears to be orthologous to the human sequence, supporting the composition of the human consensus sequence. However the mouse prediction had extra sequence at the 3' end, making it complete. Also the first exon of the mouse sequence  
30 did not match the human sequence, and may have resulted from a GENSCAN error or artefact.

#### Example 2

##### **Expression of Human ADAMTS-E in Normal and Diseased Tissues**

A panel of human cDNA libraries was screened using PCR primers designed from the  
35 ADAMTS-E sequence (Forward: 5'- GACATCTGCATCTACAAG-3' [SEQ ID NO: 5] and Reverse

5'-GAATGGGTTGGTCTTCATGG-3') [SEQ ID NO: 6] and expected to yield a product of 249 nucleotides in length. Using plasmid library DNAs (Life Technologies) as a template, ADAMTS-E was found to be present in heart, spleen, kidney, liver, brain and lung tissues. ADAMTS-E was found to be expressed in chondrocytes isolated from osteoarthritic cartilage tissue (6 of 7 patients analyzed). This was shown by preparing RNA from chondrocytes isolated from the patient samples and performing an RT-PCR analysis using the primers described above. In particular, RNA from the knee cartilage of three osteoarthritis patients (FIG. 5, lanes 10, 11, and 12) treated with (+) or without (-) retinoic acid and oncostatin M was used to synthesize cDNA for amplification in PCR with primers for ADAMTS-E. A heart cDNA library (H) was employed as positive control. The expected 247bp PCR product was weakly detected in all three patient samples, and no induction was observed with retinoic acid and oncostatin M stimulation.

The foregoing description of the invention has been presented for purposes of illustration and description. Further, the description is not intended to limit the invention to the form disclosed herein. Consequently, variations and modifications commensurate with the above teachings, and the skill or knowledge in the relevant art are within the scope of the present invention. It is intended that the appended claims be construed to include alternate embodiments to the extent permitted by the prior art.